



## Research paper

## Application of Hansen Solubility Parameters to predict drug–nail interactions, which can assist the design of nail medicines

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## ABSTRACT

We hypothesised that Hansen Solubility Parameters (HSPs) can be used to predict drug–nail affinities. Our aims were to: (i) determine the HSPs ( $\delta_D$ ,  $\delta_P$ ,  $\delta_H$ ) of the nail plate, the hoof membrane (a model for the nail plate), and of the drugs terbinafine HCl, amorolfine HCl, ciclopirox olamine and efinaconazole, by measuring their swelling/solubility in organic liquids, (ii) predict nail–drug interactions by comparing drug and nail HSPs, and (iii) evaluate the accuracy of these predictions using literature reports of experimentally-determined affinities of these drugs for keratin, the main constituent of the nail plate and hoof. Many solvents caused no change in the mass of nail plates, a few solvents deswelled the nail, while others swelled the nail to varying extents. Fingernail and toenail HSPs were almost the same, while hoof HSPs were similar, except for a slightly lower  $\delta_P$ . High nail–terbinafine HCl, nail–amorolfine HCl and nail–ciclopirox olamine affinities, and low nail–efinaconazole affinities were then predicted, and found to accurately match experimental reports of these drugs' affinities to keratin. We therefore propose that drug and nail Hansen Solubility Parameters may be used to predict drug–nail interactions, and that these results can assist in the design of drugs for the treatment of nail diseases, such as onychomycosis and psoriasis. To our knowledge, this is the first report of the application of HSPs in ungual research.

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## 1. Introduction

The human nail can suffer from a number of diseases, such as onychomycosis (fungal infections of the nail) and psoriasis [1]. These diseases are common and significantly affect the sufferers' quality of life; however, their current treatment is far from ideal, with long treatment durations and low success rates, even for the newest medicines [2–9]. For effective treatment, drugs must permeate into the nail (their site of action) following topical and/or oral administrations. Ungual (i.e. of the nail) drug permeation depends on the properties of the drug (such as its size, charge), the formulation (such as its pH, which influences drug charge), the nail plate (such as its porosity and hydration), as well as drug–nail, drug–formulation and formulation–nail interactions, such as drug–keratin binding, drug release from the formulation and the latter's adhesion to and residence on the nail plate. The influence of these factors on ungual permeability has been researched to varying extents, and the need for a more rational approach to the development of new therapies is obvious [10].

While the relationship between certain factors and ungual permeation is well-established (for example that of drug size) [11,12], the influence of other parameters such as nail–drug interactions, has been much less investigated.

Drug binding to keratin – the main constituent of the human nail plate – is known to reduce its antifungal effect, as shown by Tatsumi et al. (2002) who reported a direct relationship between a drug's affinity to keratin and the extent of reduction in its antifungal activity in the presence of keratin [13]. Drug binding to nail keratin is also expected to influence ungual drug permeation. For example, in evaluations of topical drug carriers in Franz cell type setups, a high affinity for nail keratin is expected to lead to greater drug partitioning out of the carrier into the nail plate. Concurrently, a high drug–nail affinity will reduce the drug partitioning out of the nail into the receptor medium, resulting in lower drug flux and calculated ungual permeability. This may help explain the lower flux, but greater drug-in-nail levels of terbinafine (which has higher keratin affinity than amorolfine [13]) when UV-cured gels loaded with terbinafine or amorolfine were evaluated as topical nail medicines [14]. Sugiura et al. (2014) also suggested that the greater ungual permeability of efinaconazole, compared to amorolfine and ciclopirox, was related to its lower keratin affinity [15].

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The ability to predict drug–nail interactions will therefore assist effective drug design and the success of therapy. With this in mind, the aims of the research discussed in this paper were to:

- (i) Determine the Hansen Solubility Parameters (HSP) of the nail plate, the hoof membrane (a commonly used model for the nail plate in ungual research due to the scarcity and expense of nail plates), and of the anti-onychomycotic drugs terbinafine HCl, amorolfine HCl, ciclopirox olamine and efinaconazole.
- (ii) Predict nail–drug interactions based on the determined HSPs, and evaluate whether these predictions reflect literature reports of experimentally-determined affinities of these drugs for keratin.

Solubility parameters – first developed by Hildebrand and co-workers [16], and which help to quantify the statements ‘like dissolves like’ or ‘like seeks like’ [16–18], have been widely used to predict materials’ compatibilities, including in pharmaceutical drug development [19–22]. Hansen Solubility Parameters divide the total solubility parameter ( $\delta_T$ ) into individual parts arising from dispersion forces ( $\delta_D$ ), permanent dipole–permanent dipole forces ( $\delta_P$ ), and hydrogen bonding ( $\delta_H$ ) [17] such that:

$$\delta_T^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \quad (1)$$

The HSP parameters of materials can be found experimentally, based on the observation of interaction (or its absence) between the test material and solvents with well-defined HSPs. The solvents are then divided into those which interact strongly with the test material (‘good’ solvents), and those which do not interact (‘bad’ solvents). The  $\delta_D$ ,  $\delta_P$ ,  $\delta_H$  HSPs of all the solvents are then plotted 3-dimensionally, and a computer program locates the ‘sphere’ in HSP space that includes the ‘good’ solvents and excludes the ‘bad’ solvents, with a minimum of error. The centre coordinates of the sphere give the HSPs ( $\delta_D$ ,  $\delta_P$ ,  $\delta_H$ ) of the test material, while the radius of the sphere ( $R_o$ ) describes how large/small the interaction range is.

To compare two materials,  $R_a$  (the solubility parameter ‘distance’) between them can be calculated by

$$(R_a)^2 = 4(\delta_{D2} - \delta_{D1})^2 + (\delta_{P2} - \delta_{P1})^2 + (\delta_{H2} - \delta_{H1})^2 \quad (2)$$

where the subscripts 1 and 2 refer to material 1 and material 2.

For high affinity between two materials,  $R_a$  must be less than the  $R_o$  of the test material, and a Relative Energy Difference (RED) value is often used to quantify distances  $R_a$  relative to  $R_o$  as follows:

$$RED = \frac{R_a}{R_o} \quad (3)$$

Thus  $RED < 1$  indicates high affinity, while  $RED > 1$  indicates low affinity.

In applying this approach to the nail, knowledge of the nail plate’s HSPs and use of drug–nail RED values is expected to assist the design and/or selection of drugs which have the desired (high or low) affinity for the nail plate. A high drug–nail affinity may mean that an antifungal agent’s potency is diminished, but it also means that the drug will concentrate in the nail, resulting in a drug depot in the nail which can enable less frequent drug dosing, as evidenced by oral itraconazole pulse therapy in the treatment of onychomycosis. The nail plate’s HSPs will also be useful in other industries, such as the nail cosmetic industry, where various chemicals are used, and where chemical–nail interactions are important for the cosmetic’s properties such as wear. Knowledge of the hoof membrane’s HSPs should help us further evaluate this material’s suitability as a model for the nail plate in ungual research.

## 2. Materials and methods

### 2.1. Materials

Healthy nail clippings were collected from adult female and male volunteers, aged 15–45 years (ethics approval, REC/B/10/01 School of Pharmacy, University of London, UK). Bovine hoof membranes were obtained from Madras Veterinary College Teaching Hospital (Chennai, India). Amorolfine hydrochloride was obtained from Ranbaxy Research Laboratories (Gurgaon, Haryana, India). Terbinafine hydrochloride was obtained from AK Scientific (Union City, CA, USA). Ciclopirox olamine was obtained from Zhejiang Huade Chemicals Co. (Taizhou, Zhejiang, China).

Butyl benzoate, decane, dibutyl phthalate, 1,5-dichloropentane, dimethyl phthalate, 2-ethyl hexanol, glycerol triacetate, 1-hexene, octane, 1-octanol, triethyl citrate, 1,2-propanediol, butanol, 1-methyl-2-pyrrolidone, 1-octanol, 1-pentanol, 1-vinyl-2-pyrrolidone, 2,2,4-trimethylpentane, acetone, acetic anhydride, butyl acetate, diethylamine, ethanolamine, diethanolamine, dimethyl formamide, dimethyl sulfoxide, ethylene glycol, formamide, isopropyl myristate, isopropyl palmitate, piperidine, pyridine, diethyl ether, dibutyl phthalate, benzyl butyl phthalate, acetophenone, propylene carbonate, methyl acetate, ethyl acetate, diacetone alcohol, dodecanol, methyl formate, 2-chloroethanol, hexanol, pyrrole, acetaldehyde, 4-chloro-1-butanol, 3-chloro-1-propanol, ethylenediamine, amino-2-propanol, 2-methylamino propanol, 1-methoxy-2-propanol, were obtained from Sigma Aldrich (UK). Acetone, acetonitrile, benzaldehyde, formaldehyde, hexane, methanol, and triethylamine were obtained from Fisher Scientific (Loughborough, UK). Lactic acid was obtained from Fisons Scientific Equipment (London, UK). Heptane was obtained from BDH Chemicals Ltd (UK). Glycerol was obtained from Alfa Aesar (Heysham, UK).

### 2.2. Methods

#### 2.2.1. Determination of nail and hoof HSPs

Sample preparation: Fingernail and toenail clippings were washed with distilled water and any debris was carefully removed using a spatula without damaging the nail plate. The nail plates were then allowed to dry and equilibrate at room temperature for at least 60 min. The relative humidity in the laboratory was on average about 44%, where, the nail water content is expected to be about 8% w/w (from relative humidity – nail water content profiles reported in [23–26]). While it is possible that the nail’s water content could affect the extent of swelling by organic liquids, we opted to air-dry (as opposed to oven-dry) the nails, as the data obtained in this study are more likely to reflect real-life situation (where nails are hydrated to some extent). Slices of bovine hoof membranes were cut from larger pieces using a scalpel, following overnight soaking in water to soften the tissue. The hoof slices were cut into a thickness of approximately 0.40 mm to reflect that of human nail clippings; following measurement of twenty finger- and toe-nail clippings from different donors using a digital micrometre, the average thicknesses were found to be 0.34 mm and 0.5 mm for finger- and toe-nail clippings respectively.

Nail and hoof HSPs were determined by measuring their swelling behaviour in a selection of solvents, of varying and well-defined HSP values. Fingernail and toenail clippings and hoof slices were weighed, then placed individually in glass vials, after which three mL of a solvent was added, and the vials were placed in a water bath at 25 °C. The mass of the nail clipping/hoof was monitored at regular intervals for a total of 35 days, by removing the sample, wiping off the excess liquid and weighing the sample before returning to the vial. The experiments were carried out in

triplicate. The mean extent of nail swelling for each solvent was then inputted into the HSPiP® software (3rd edition 3.1.12). The software plots the  $\delta_D$ ,  $\delta_P$  and  $\delta_H$  of each organic liquid along the three axes of a three-dimensional graph, after which, the program calculates a sphere whose centre coordinates give the  $\delta_D$ ,  $\delta_P$  and  $\delta_H$  of the nail/h hoof. Multiple calculations allowed the obtainment of mean and standard deviation values. Only solvents which did not damage the nail plate and hoof were used for the HSP calculation, i.e. phosphoric acid, lactic acid, ethanolamine and ethylene diamine were excluded. Water was also excluded from the HSP calculation, due to its exceptional behaviour as a solvent, and as per [27]. Due to the small influence of solvent molar volume on nail swelling, HSPs were also calculated using only solvents with molar volumes between 50 and 100 cm<sup>3</sup>/mol. This did not influence the HSP values or the fit greatly (data not shown), and therefore the values shown in Table 2 are those using solvents with molar volumes 37–330 cm<sup>3</sup>/mol. HSPs were also calculated using double spheres in the HSPiP software. This did not result in any improvement of the fit, and the HSPs reported in this paper are therefore those using single spheres.

### 2.2.2. Determination of antifungal drug HSPs

The solubilities of terbinafine hydrochloride, amorolfine hydrochloride, and ciclopirox olamine in a number of solvents with a wide range of HSPs were investigated. The number of solvents used (between 33 and 39 for each drug) depended on the number needed to optimise the HSP sphere fit to be as close as possible to 1, and fits of 0.9–1.0 were obtained. Rough saturation solubility testing was carried out whereby 10 mg of each drug was added to one mL of each solvent, and the mixtures were left stirring overnight in a water bath at 25 °C, followed by visual inspection to determine whether the drug had fully dissolved. If the drug had fully dissolved, another 10 mg of drug was added, the mixture stirred overnight and inspected. The process was repeated until drug precipitation was seen or until a total of 100 mg of drug had been added. Finally, the solutions were left in the water bath at 25 °C for

**Table 2**

HSPs of fingernail, toenail and hoof pieces.

Sample	HSPs (MPa <sup>1/2</sup> )				Ro
	$\delta_D$	$\delta_P$	$\delta_H$	Total ( $\delta_T$ )	
Fingernail	17.7 ± 0.5	20.9 ± 0.3	18.6 ± 0.6	27.4 ± 0.2	12.7 ± 0.4
Toenail	15.9 ± 0.3	19.6 ± 0.7	19.3 ± 0.4	25.3 ± 0.6	11.5 ± 0.6
Hoof	16.5 ± 0.5	15.5 ± 1.2	19.3 ± 1.4	23.5 ± 2.1	10.5 ± 2.3

72 h to ensure that no change in drug solubility occurred. Each solvent was then given either a score of 1 if 10 mg or more of the drug dissolved, or a score of zero if 10 mg did not dissolve. The solubility scores were then inputted into the HSPiP software, which calculated the drugs' HSPs.

Two additional solubility scoring methods were used to determine the HSPs of ciclopirox olamine, in order to determine which method would give an optimal solubility sphere and fit. The first was assignment of scores of 1–6 to each solvent as follows: scores of 1, 2, 3, 4, 5 and 6 for drug dissolution of: ≥80 mg, <80 and ≥50 mg, <50 and ≥40 mg, <40 and ≥20 mg, <20 and ≥10 mg, <10 mg respectively. The second was the assignment of a score according to the total amount of drug dissolved in each solvent. For instance, if 50 mg of ciclopirox was dissolved in a solvent, then the score would be 50 for that solvent. These two additional scoring methods were not as practical and did not improve data fits compared to the first method mentioned in the paragraph above, and were therefore not explored further.

The HSP of efinaconazole was obtained from the HSPiP software, due to the high cost of the amount of efinaconazole needed for solubility studies and the fact that efinaconazole's HSP can be calculated (unlike those of the drug salts). The  $\delta_T$ ,  $\delta_D$ ,  $\delta_P$ , and  $\delta_H$  values were calculated from the molecular formula, using the Y-MB method. The latter was developed by fitting thousands of compounds using Neural Network techniques and enables the estimation of a chemical's properties (HSPiP software eBook).

### 2.2.3. Validation of the measured HSPs

The experimentally determined HSP values should enable qualitative predictions of nail/h hoof swelling and of drug solubility in any untested liquid if the latter's HSPs are known. In order to determine whether this holds true, nail/h hoof swelling and drug solubility in a number of solvents – which had not been used in the HSP determination – were theoretically predicted, and the accuracy of the predictions was then experimentally tested as described in Sections 2.2.1 and 2.2.2. Theoretical predictions were based on RED values as explained in the Introduction, with a RED value of less than one predicting nail/h hoof swelling or drug dissolution in a liquid at a concentration of 10 mg/mL.

The liquids used for the validation of nail/h hoof swelling are shown in Table 4, and had molar volumes under 100 m<sup>3</sup>/mol to avoid any possible effects of molecular size on swelling. Of these, five were predicted to swell nail, and four were predicted to cause no swelling. The liquids used for the validation of drug solubility are shown in Table 5; approximately half of the drug-liquid combinations were predicted to show drug solubility. HSPs of all the liquids were obtained from the HSPiP software.

**Table 3**

HSPs of amorolfine HCl, terbinafine HCl, ciclopirox olamine, and efinaconazole.

Drug	HSPs (MPa <sup>1/2</sup> )			
	$\delta_D$	$\delta_P$	$\delta_H$	$\delta_T$
Amorolfine HCl	18.1 ± 0.4	12.2 ± 0.5	16.9 ± 0.3	22.6 ± 0.4
Terbinafine HCl	17.1 ± 0.7	16.6 ± 1.1	13.9 ± 0.6	23.8 ± 1.4
Ciclopirox olamine	17.4 ± 0.6	13.9 ± 0.6	17.2 ± 0.6	28.1 ± 0.3
Efinaconazole	19.1	6	5.6	20.8

**Table 1**

% Mass change of finger nails, toe nails and hoof membranes, following a 35-day incubation in liquids,  $n = 3$ .

Liquid	Fingernail	Toenail	Hoof
Phosphoric acid	155.0 ± 18.8	120.0 ± 16	Destroyed
Ethanolamine	142.2 ± 14.5	93.4 ± 3.3	Destroyed
Ethylene diamine	145.0 ± 4.0	154.0 ± 12	Destroyed
Lactic acid	88.4 ± 2.6	75.8 ± 6.0	186 ± 20
Water	55.0 ± 0.8	43.3 ± 4.1	61.2 ± 0.4
Formamide	53.3 ± 2.9	56.7 ± 2.5	88.3 ± 14.4
DMSO	52.3 ± 7.9	48.3 ± 9.2	93.1 ± 17.5
2-Chloroethanol	48.4 ± 7.3	53.2 ± 11.8	76.3 ± 4.3
Ethylene glycol	37.2 ± 6.4	31.0 ± 5.3	73.0 ± 13
Formaldehyde	26.8 ± 7.4	31 ± 2.5	43.0 ± 1.1
2-Methoxy ethanol	16.4 ± 0.6	13.9 ± 10.4	30.5 ± 2.8
Amino-2-propanol	14.7 ± 4.6	14.0 ± 1.8	29.7 ± 2.8
2-Methyl amino propanol	12.8 ± 2.1	15 ± 10.7	25.3 ± 1.2
DMF	11.2 ± 3.5	18.4 ± 2.1	34.3 ± 8.8
Methanol	11.2 ± 3.5	8.1 ± 4.5	29.7 ± 22.2
Dichloromethane	8.7 ± 1.6	12.4 ± 2.9	12.7 ± 5.1
p-Xylene	8.4 ± 2.1	12.2 ± 5.1	3.2 ± 4.8
Dichloroethane	6.2 ± 2.2	6.2 ± 8.4	11.0 ± 2
NMP	5.6 ± 2.1	8.5 ± 6.0	4.2 ± 1.4
Ethanol	2.9 ± 1.7	4.0 ± 1.2	7.2 ± 2.0
Diacetone alcohol	−11.2 ± 0.8	−9.73 ± 1	−11.2 ± 5.5
Vinyl pyrrolidone	−10.9 ± 0.8	−8.9 ± 0.7	0
Benzyl alcohol	−7 ± 0.8	−5.3 ± 0.8	0
Pentanol	−5.5 ± 3.1	−4.1 ± 1.3	−1.5 ± 3.4
Acetone	−5 ± 0.4	−5.12 ± 0.7	0
Toluene	−8.3 ± 2.1	0	−4.7 ± 4.6
Octanol	0	0	−11.7 ± 1.5
Butanol	0	0	−1.5 ± 0.9

**Table 4**

Predicted and experimentally-determined nail and hoof swelling in liquids.

Liquid	RED between liquid and nail/hoof			Nail/hoof swells?	
	Fingernail	Toenail	Hoof	Predicted	Experimental
N-methyl formamide	0.7	0.7	0.4	Yes	Yes
Methyl iodide	1.5	1.2	0.9	<b>Nails no; hoof yes</b>	<b>Yes</b>
Ethylene cyanohydrin	0.8	0.7	0.5	Yes	Yes
Glycerol formal	0.6	0.6	0.7	<b>Yes</b>	<b>No</b>
1,4-Butanediol	1.0	1.1	1.0	No	No
Pyrrole	0.7	0.8	0.7	Yes	Yes
Allyl alcohol	0.7	0.6	0.5	Yes	Yes
Carbon disulphide	2.2	1.2	1.8	No	No
Biuret	1.0	1.4	1.1	No	No

Bold text shows disagreement between predicted and experimental swelling.

#### 2.2.4. Prediction of nail–drug affinities based on calculated HSPs, and comparisons with literature values of experimentally-determined drug–keratin affinities

Nail–drug RED values for terbinafine HCl, amorolfine HCl, ciclopirox olamine and efinaconazole were calculated using the following equation:

$$\text{RED} = \frac{Ra}{Ro} \quad (4)$$

where  $Ra$  is the solubility parameter ‘distance’ between the nail and the drug, and was calculated using Eq. (2) (shown in the Introduction) and  $Ro$  is the radius of the nail’s HSP sphere. RED values below 1 were taken as indications of high nail–drug affinities. Conversely, RED values above 1 were taken as indications of low nail–drug affinities. The predicted nail–drug affinities were then compared with experimentally-obtained values of drug–keratin affinities, which were obtained from Sugiura et al. [15] and Tatsumi et al. [13].

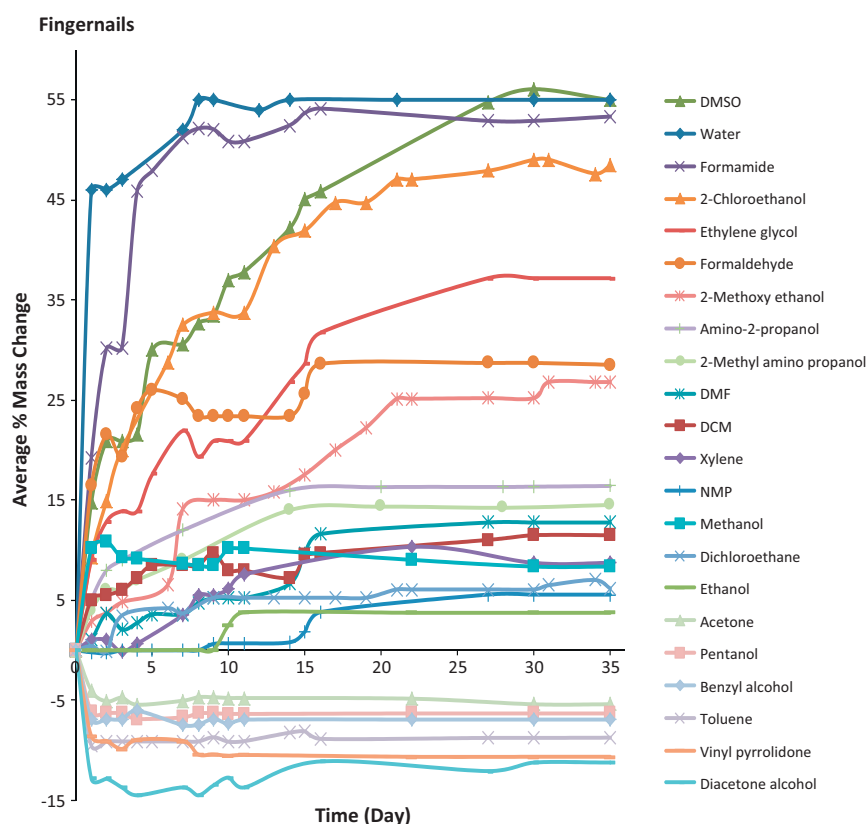
### 3. Results and discussion

With a few exceptions, the liquids which swelled or deswelled (i.e. caused an increase or decrease in nail/hoof mass) the fingernails, also swelled toenails and hoof pieces, and those which caused no change in the mass of fingernails also caused no change in the mass of toenails and hoof pieces (Table 1).

#### 3.1. Swelling behaviour of nail and hoof in a range of organic liquids

##### 3.1.1. No nail swelling

A large number of liquids – more than half of those tested – caused no change in the mass of fingernail, toenail and hoof, that is, showed limited affinity for, and uptake into the nail/hoof. These liquids include acetic anhydride, acetonitrile, acetophenone, benzaldehyde, benzyl butyl phthalate, 4-chloro-butanol, chlorobenzene, chloroform, 3-chloropropanol, cyclohexane, cyclohexanol,



**Fig. 1.** % Mass change of fingernail clippings incubated in organic liquids over time. Mean of  $n = 3$  is shown. Solvents which caused no change in mass and standard deviations are not shown to ease visualisation.

dibutyl phthalate, 1,3-dioxolane, dodecanol, ethyl acetate, glycerol, glycerol triacetate, hexane, 1-hexanol, isopropyl palmitate, 1-methoxy-2-propanol, piperidine, propylene carbonate, propylene glycol, and THF. The lack of nail swelling is likely due to the fact that the nail plate has a highly ordered, thus stable, structure in its component proteins and lipids. The nail plate consists mainly of keratin, followed by water (at 8–22% depending on ambient humidity), lipids (which make up less than 5% of the nail plate mass) and small amounts of elements such as calcium, magnesium, sodium, potassium, iron, copper, zinc, aluminium, and chlorine [28–30]. The nail plate proteins are known to be predominantly in the  $\alpha$ -helix conformation, heavily folded and linked by multiple disulphide bonds which are also in a stable conformation, while the lipids are in a highly-ordered crystal state and much of the nail plate water content is bound to keratin [31,32].

### 3.1.2. Nail swelling

Liquids which swell/deswell the nail/h hoof are shown in Table 1, and the swelling/deswelling profiles are shown in Figs. 1–3. The extents of fingernail and toenail swelling in a particular liquid at day 35 were statistically the same (ANOVA and post hoc Tukey;  $p > 0.05$ , Table 1), although swelling occurred at a faster rate in fingernails, possibly due to their thinner structure. Hoof pieces swelled to greater extents compared to fingernails and toenails (ANOVA and post hoc Tukey;  $p \leq 0.05$ , Table 1), correlating with literature reports of its greater swelling in water [33] and its greater permeability [12].

The extent of nail/h hoof swelling was significantly different for the different liquids (Table 1). One way ANOVA and post hoc Tukey on the extent of swelling at day 35 showed that, for both fingernails and toenails, solvents which swelled (but did not destroy) the nail plates can be grouped in descending order of % swelling as follows: (i) water, formamide, DMSO and 2-chloroethanol, (ii) ethylene glycol and formaldehyde, and (iii) ethanol, methanol, 2-amino-propanol, DCM, dichloroethane, 2-methoxy ethanol, 2-methyl amino propanol, NMP, xylene and DMF. The groups differed significantly ( $p \leq 0.05$ ) from one another in terms of extent of nail swelling, while no statistically significant difference was obtained among solvents within one group ( $p \geq 0.05$ ). Similarly, solvents which swelled the hoof slices could also be grouped in descending order of % swelling as follows: (i) DMSO and formamide, (ii) formaldehyde and ethylene glycol, and (iii) ethanol, methanol, xylene, dichloroethane, DCM, NMP, 2-methoxy ethanol, 2-methyl amino propanol and DMF (one way ANOVA and post hoc Tukey,  $p \leq 0.05$ ). Thus, it can be seen that bovine hoof and human nail plate have affinities to the same solvents, further confirming the suitability of hoof as a model for the nail.

When the properties of the organic liquids are examined for their influence on the extent of nail/h hoof swelling, a small, but significant correlation (Pearson correlation,  $p < 0.05$ ) between solvent molar volume and nail plate/h hoof swelling was found. This was expected from the literature, where a permeant's molecular size has been reported to influence its ungual permeability [11,12]. However, the correlation was small;  $r = 0.3$  for fingernails and for toenails, and  $r = 0.5$  for hoof samples, i.e. the liquid's molecular size

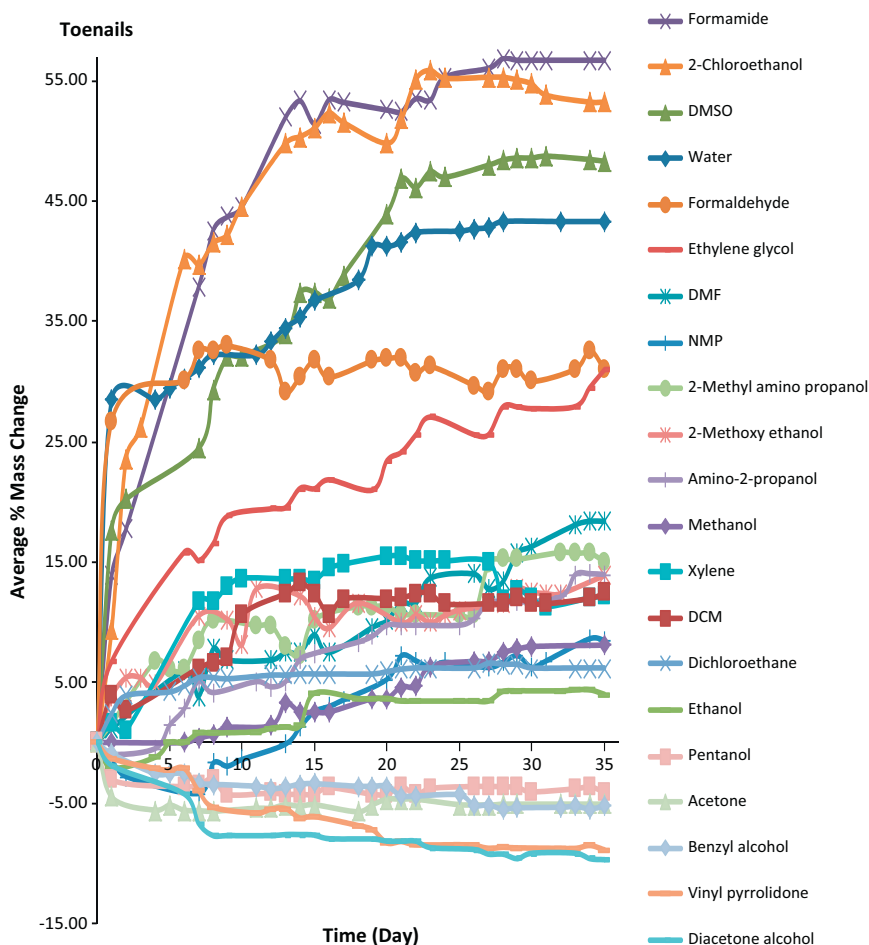
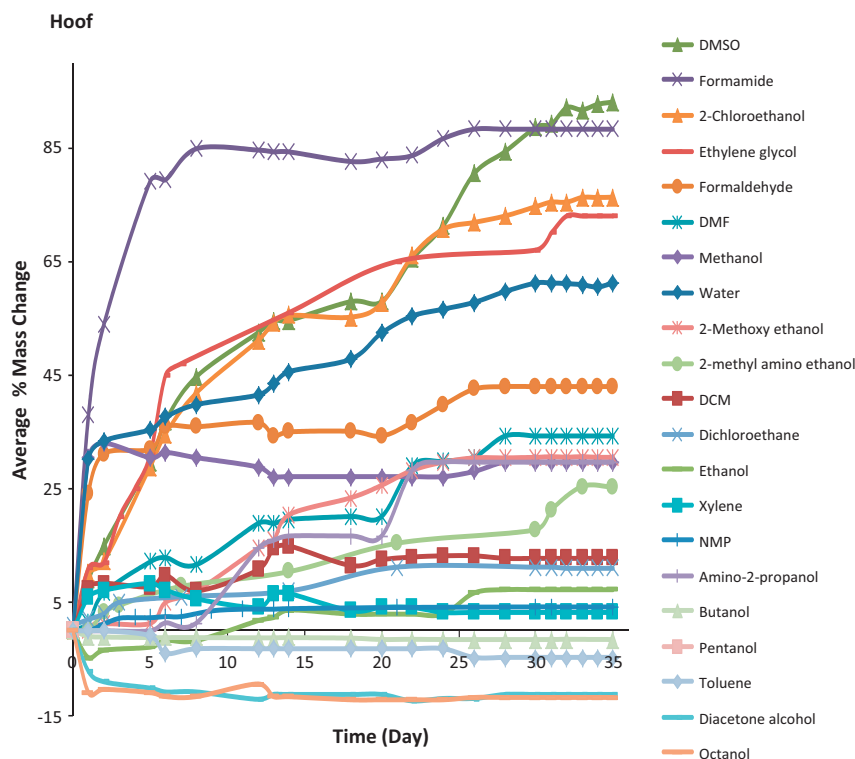


Fig. 2. % Mass change of toenail clippings incubated in organic liquids over time. Mean of  $n = 3$  is shown. Solvents which caused no change in mass and standard deviations are not shown to ease visualisation.





**Fig. 3.** % Mass change of hoof pieces incubated in organic liquids over time. Mean of  $n = 3$  is shown. Solvents which caused no change in mass and standard deviations are not shown to ease visualisation.

was not the only factor in nail/hoof swelling. When a multiple regression analysis was performed to evaluate the influence of molar volume and  $\delta_D$ ,  $\delta_P$  and  $\delta_H$  Hansen Solubility Parameters for each liquid, the  $\delta_P$  component was found to be the most important parameter that influenced nail and hoof swelling ( $p < 0.05$ ), with greater swelling in liquids with higher  $\delta_P$ . This suggests that polar drug molecules and polar liquids used as drug carriers are more likely to permeate into the nail, and that the polarities of drugs and excipients should be taken into consideration in ungual drug development.

The greatest changes to the nail/hoof sample were caused by the acids and bases ( $p < 0.05$ ), phosphoric acid, lactic acid, ethanolamine and ethylene diamine, which even destroyed the hoof membranes after causing extreme swelling. Acids and bases are known to damage the nail plate, for example, phosphoric acid and tartaric acid have been shown to etch and cause significant changes to the nail plate surface [34,35] while potassium hydroxide is often used to dissolve the nail plate, for example in the diagnosis of nail fungal infections. As expected, the stronger acid and base (phosphoric acid, ethylene diamine) caused greater swelling compared to the weaker acid and base (lactic acid and ethanolamine) respectively, although for ethylene diamine, the effect is only obvious for the toenail, possibly due to the large standard deviations. The greater swelling of the nail plate by phosphoric acid compared to lactic acid explains the greater nail plate surface modification and the greater ungual permeation enhancing effect of phosphoric acid compared to lactic acid reported by [36].

When the swelling profiles in Figs. 1–3 are examined, it can be seen that the swelling profiles seem to be liquid-specific, with similar profiles obtained for each liquid swelling fingernail, toenail and hoof. It can also be seen that the swelling profiles are significantly different for the different liquids. For example, certain liquids such as water showed a rapid increase in nail mass while other liquids,

such as ethylene glycol showed a more gradual change over the 35 days (Figs. 1–3). The different profiles of the liquids over the 35-day experiment reflect the substantially different rates of diffusion of water, propylene glycol and DMSO in the nail reported over a much shorter timescale of about a day [37]. It can also be seen from the swelling profiles that the rate of liquid uptake is not constant prior to saturation. Uptake of some liquid seems to cause changes in the nail plate, for example, opening of the keratin structure, as suggested by [38], which leads to further solvent uptake, such that liquid uptake seems to facilitate its own diffusion in the nail, as discussed in [37].

### 3.1.3. Nail deswelling

A few liquids caused deswelling of the nail and hoof (Table 1). No statistical differences in the extent of deswelling were found amongst these liquids (one way ANOVA,  $p > 0.05$ ). Deswelling (corresponding to % mass reduction of 5–15%) occurred mostly within the first day of sample incubation in a solvent (Figs. 1–3), and is expected to be due to the extraction of some of the nail plate water and/or lipid components, which account for 8–22% and less than 5% of the nail plate mass respectively [29,39,40]. This reflects previous reports of acetone causing a decrease in nail mass [33] and extracting nail water [32]. Smith et al. also proposed that organic solvents may be used to manipulate nail hydration [41]. Given the influence of nail water on its properties such as brittleness [42,43], and its enhancing effect on ungual drug permeation [44], it may be advisable to avoid those liquids (shown in Table 1) which deswell the nail in the development of nail medicines.

Nail swelling has previously been studied as a preformulation/screening tool [33,45,46] and to understand the effects of organic solvents on the barrier properties of the nail [41]. Most of the reported studies have however used aqueous solutions, and uptake

of organic solvents in the absence of water has not been extensively studied. This investigation on the behaviour of nails in a wide range of neat organic liquids shows liquids which: (i) cause no obvious change to the nail, (ii) deswell the nail and thereby may be deleterious to nail health and hinder ungual drug permeation, (iii) permeate into the nail to a great extent and could thereby drag topically-applied drugs into the nail, and (iv) are damaging to the nail, but which could act as ungual enhancers if used in lower concentrations.

### 3.2. Hansen Solubility Parameters (HSPs) of nail and hoof

Nail and hoof HSPs calculated from swelling measurements are shown in Table 2. Good fits (0.8–1.0) to the data were obtained with the HSPiP software, and it can be seen that fairly high  $\delta_p$  and  $\delta_H$  values for the nail and hoof were obtained. As a comparison, the  $\delta_D$ ,  $\delta_p$  and  $\delta_H$  of fat (lard) have been reported to be 15.9, 1.1 and 5.4 while those of blood serum have been reported to be 23.2, 22.7 and 30.6 respectively [47]. The high  $\delta_D$ ,  $\delta_p$  and  $\delta_H$  values of nail and hoof are explained by the fact that their primary constituent is the  $\alpha$ -keratin protein. The proportions of the different amino acids (number of residues per 100) in the nail plate have been found to be: lysine (3.1%), histidine (1.0%), arginine (6.4%), aspartic acid (7.0%), threonine (6.1%), serine (11.3%), glutamic acid (13.6%), proline (5.9%), glycine (7.9%), alanine (5.5%), valine (4.2%), methionine (0.7%), isoleucine (2.7%), leucine (8.3%), tyrosine (3.2%), phenylalanine (2.5%), and half cystine (10.6%) while the sulphur content was 3.2% of the nail plates' dry weight [24]. The amino acid proportions in bovine hoof are fairly similar, with the proportions (number of residues per 100) being: lysine (4.3%), histidine (0.8%), arginine (7.1%), aspartic acid (8.9%), threonine (4.6%), serine (8.3%), glutamic acid (15.8%), proline (3.2%), glycine (9.4%), alanine (7.2%), valine (5.8%), methionine (0.5%), isoleucine (3.9%), leucine (10.4%), tyrosine (2.9%), phenylalanine (2.6%), and half cystine (4.3%) [48]. The high  $\delta_p$  and  $\delta_H$  values of the nail and hoof in Table 2 reflect the nature of the keratin protein, with its large number of polar amino acid residues and hydrogen bonds which stabilise the protein. The high  $\delta_p$  and  $\delta_H$  values also explain the finding that the nail plate behaves like a 'hydrogel' [49].

The HSPs of the nail and hoof were fairly similar, except for a slightly lower  $\delta_p$  of the hoof (Table 2). The similarities between nail and hoof HSPs reflect their similar compositions as described above. It has also been said that the 'corresponding keratin species of cow and human are, by and large, highly conserved in their charge, size and immunoreactivities' [50]. Mertin and Lippold showed almost parallel plots for the nail plate and hoof membrane, when permeability coefficient was plotted against permeant molecular weight [12], while Murdan et al. showed similar surface energies of human nail and bovine hoof [51] and Nicoli et al. showed that human nail and bovine hoof had the same isoelectric point, which was close to 4 [52]. The similar HSPs between nail and hoof show that the interaction of a solvent with a nail may be predicted by its interaction with hoof. Thus, the latter can serve as a model for the scarce and expensive nail plate in certain experiments. The slightly lower  $\delta_p$  of the hoof compared to the nail could be due to the slight differences in the proportions of the different amino acids, for example, the nail being richer in the polar cysteine.

As expected, the values of  $\delta_D$ ,  $\delta_p$  and  $\delta_H$  were similar for fingernails and toenails (Table 2). While certain differences between fingernails and toenails have been reported, for example, their dehydration profiles, growth rates, thickness, concentration of calcium and magnesium, surface pH, transonycheal water loss, Raman spectra and different susceptibilities to fungal infections [30,53–60], fingernails and toenails are, by and large, almost the same material, which is shown by their same  $\delta_D$ ,  $\delta_p$  and  $\delta_H$ .

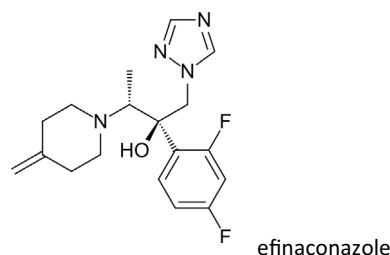
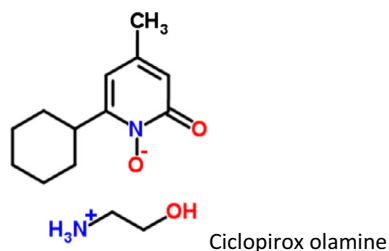
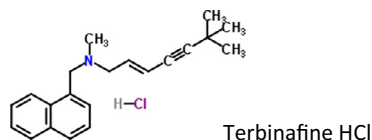
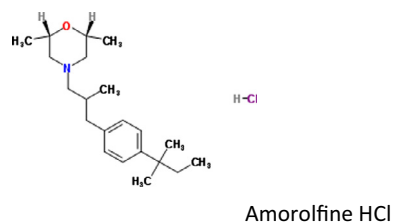


Fig. 4. Chemical structures of amorolfine HCl, terbinafine HCl, ciclopirox olamine and efinaconazole.

### 3.3. HSPs of the terbinafine HCl, amorolfine HCl, ciclopirox olamine and efinaconazole

The estimated HSPs and chemical structures of the anti-onychomycotic drugs are shown in Table 3 and Fig. 4 respectively. The salts terbinafine HCl, amorolfine HCl and ciclopirox olamine have high polar and hydrogen bonding parameters in contrast to efinaconazole. Knowledge of the HSPs of these drugs enables one to understand the potential interactions between the nail and these drugs. In addition, the known drug HSPs can also be used in the rational design of these drugs' formulations [61].

### 3.4. Validation of the HSP measurements

To verify that the experimentally determined HSPs shown in Tables 2 and 3 may be used for predictions, nail/hoof swelling and drug solubility in a number of previously unused liquids were predicted and experimentally tested. The results are shown in Tables 4 and 5. It can be seen that, of the nine liquids tested for nail and hoof swelling, agreement between predicted and experimental swelling was found for seven liquids for fingernails and toenails, and for eight liquids for the hoof. The predictions were correct for 82% of the nail/hoof and liquid combinations. Meanwhile, the

**Table 5**

Predicted and experimentally-determined solubilities of the three drugs in liquids.

Liquid	Ciclopirox olamine		Amorolfine HCl		Terbinafine HCl	
	Drug/liquid RED	Solubility predicted; experimentally-determined	Drug/liquid RED	Solubility predicted; experimentally-determined	Drug/liquid RED	Solubility predicted; experimentally-determined
Pyrrole	0.5	Yes; yes	0.6	Yes; yes	0.7	Yes; yes
Allyl alcohol	0.4	Yes; yes	0.4	Yes; yes	0.4	Yes; yes
Benzyl butyl phthalate	1.03	No; no	1.2	No; no	1.1	No; no
Dibutyl phthalate	1.04	No; no	1.2	No; no	1.1	No; no
Propylene glycol	0.5	Yes; yes	0.5	Yes; yes	0.6	Yes; yes
Propanol	0.5	Yes; yes	0.7	Yes; yes	0.7	Yes; yes
Piperidine	0.8	Yes; yes	1.0	No; no	1.1	No; no

**Table 6**

Drug–nail interactions: HSP-based predictions and literature-based confirmation.

Drug	HSP-based		Literature reports of drug-keratin interaction			
	Drug–nail RED value for fingernail; toenail; hoof	Predicted interaction between drug and nail/hoof	Sugiura et al. [15]		Tatsumi et al. [13]	
			% free drug <sup>a</sup>	% drug release from keratin <sup>b</sup>	% drug bound to keratin <sup>c</sup>	% drug release from keratin <sup>d</sup>
Amorolfine HCl	0.7; 0.8; 0.5	Yes	1.9 ± 0.2	6.9 ± 0.1	92	39
Terbinafine HCl	0.5; 0.6; 0.5	Yes	ND	ND	96	22
Ciclopirox olamine	0.6; 0.6; 0.3	Yes	0.7 ± 0.0	2.4 ± 0.2	ND	ND
Efinaconazole	1.6; 1.8; 1.7	No	14.3 ± 0.4	46.0 ± 0.6	63	67

ND-not determined.

<sup>a</sup> % of free drug measured following the incubation of drug with keratin in Tris–HCl buffer at 37 °C for 1 h.<sup>b</sup> % drug release from drug-loaded keratin after 5 washes.<sup>c</sup> % of drug bound to keratin measured following the incubation of drug with keratin in saline at 37 °C for 1 h.<sup>d</sup> % drug release from drug-loaded keratin after 10 washes.

prediction of drug solubilities was correct at 100%. Such high correlations between prediction and experimental finding confirm that the HSPs shown in Tables 2 and 3 can be used as a guide to select liquids for use in, for example, nail cosmetics and drug formulation, based on the liquids' HSPs. The latter are available for a very large number of liquids, for example, in the HSPiP software and in [27]. The less than 100% correlation between the predicted and experimentally-determined swelling of nail/hoof is not unexpected. During HSP determination, a few solvents are sometimes incorrectly placed outside or inside of the HSP sphere, when the latter is being fitted; hence, the fit is less than 1.0.

### 3.5. Prediction of drug–nail interactions using HSPs

Calculated drug–nail RED values and related predictions of drug–nail affinities are shown in Table 6, along with literature-based values of keratin–drug interactions. Amorolfine HCl, terbinafine HCl and ciclopirox olamine are predicted to have high affinities for the nail and hoof (as their nail–drug RED values are less than 1), while efinaconazole is predicted to have low affinity (nail–drug RED being higher than 1). These predictions perfectly match literature reports of these drugs' interactions with keratin, as shown in Table 6. When amorolfine HCl, terbinafine HCl and ciclopirox olamine were incubated with keratin powder, most of the drug became bound to the keratin, and subsequent washing of the drug-loaded keratin resulted in low levels of drug release [13,15]. In contrast, a much smaller proportion of efinaconazole became bound to keratin, and subsequent washing of the drug-loaded keratin resulted in greater levels of drug release.

It can also be seen from Table 6 that drug–nail/hoof RED values (Table 6) for fingernail, toenail and hoof are fairly similar, being either above 1 (for efinaconazole) or below 1 (for the other three drugs). This is due to their HSPs being similar (as shown in Table 2) and indicates that hoof pieces may be used

as a model for the nail plate when drug–nail interactions are being investigated.

## 4. Conclusions

We report the swelling behaviour of nails in a wide range of organic liquids. A number of organic liquids cause no obvious change to the nail plate, others deswell the nail and may thereby adversely influence its health and its permeability, others swell and could drag permeant into the nail plate, while others are damaging to the nail but could possibly be used as ungual enhancers at low concentrations. The nail plate and hoof showed similar HSPs and high polar and hydrogen-bonding parameters, reflecting their principal constituent of keratin. As expected fingernail and toenail HSPs were almost the same, while hoof HSPs were similar to nail HSPs, indicating their usefulness as models for the nail plate. The HSPs of the anti-onychomycotic drugs terbinafine HCl, amorolfine HCl and ciclopirox olamine were also experimentally determined, while that of efinaconazole was calculated using the HSPiP software.

We showed that nail and drug HSP values can be used to predict the nail swelling and drug solubility respectively, in liquids whose Hansen Solubility Parameters are known. Furthermore, we showed that HSPs can be used to predict drug–nail affinities. High affinities between the nail/hoof and amorolfine HCl, terbinafine HCl and ciclopirox olamine and low efinaconazole–nail affinity were predicted, and these predictions were found to accurately match experimentally-determined literature reports of the binding characteristics of these drugs to keratin. Predicted drug–nail and drug–hoof interactions were the same, indicating the applicability of the hoof as a model for the nail plate in such experiments. Finally, we propose that the reported values of nail HSPs may be used to predict the nail's interactions with other drugs, which could assist the design of new drugs for the treatment of nail diseases, such as onychomycosis and psoriasis.



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## References

- [1] P. Rich, R.K. Scher, An atlas of diseases of the nail, in: *The Encyclopedia of Visual Medicine Series*, The Parthenon Publishing Group, London, 2003.
- [2] M.M.A. Elsayed, Development of topical therapeutics for management of onychomycosis and other nail disorders: a pharmaceutical perspective, *J. Controlled Release* 199 (2015) 132–144.
- [3] S. Murdan, Focal drug delivery to the nail, in: A.J. Domb, W. Khan (Eds.), *Focal Controlled Drug Delivery*, Springer, New York, 2014, pp. 561–584.
- [4] A.K. Gupta, F.C. Simpson, New pharmacotherapy for the treatment of onychomycosis: an update, *Expert Opin. Pharmacotherapy* 16 (2) (2015) 227–236.
- [5] Y. Oram, A.D. Akkaya, Treatment of nail psoriasis: common concepts and new trends, *Dermatol. Res. Practice* (2013). 13 pages. Article ID 180496.
- [6] A. Kushwaha et al., Emerging therapies for the treatment of ungual onychomycosis, *Drug Dev. Ind. Pharm.* 41 (10) (2015) 1575–1581.
- [7] H.N. Shivakumar et al., Ungual and transungual drug delivery, *Drug Dev. Ind. Pharm.* 38 (8) (2012) 901–911.
- [8] M.V. Saner, A.D. Kulkarni, C.V. Pardeshi, Insights into drug delivery across the nail plate barrier, *J. Drug Targeting* 22 (9) (2014) 769–789.
- [9] B.E. Elewski et al., Efinaconazole 10% solution in the treatment of toenail onychomycosis: two phase III multicenter, randomized, double-blind studies, *J. Am. Acad. Dermatol.* 68 (4) (2013) 600–608.
- [10] M.B. Delgado-Charro, A pharmaceutical perspective on drug delivery to the nail: recent advances and challenges, *Ther. Delivery* 6 (7) (2015) 773–775.
- [11] Y. Kobayashi et al., In vitro permeation of several drugs through the human nail plate: relationship between physicochemical properties and nail permeability of drugs, *Eur. J. Pharm. Sci.* 21 (4) (2004) 471–477.
- [12] D. Mertin, B.C. Lippold, In-vitro permeability of the human nail and of a keratin membrane from bovine hooves: prediction of the penetration rate of antimicrobials through the nail plate and their efficacy, *J. Pharm. Pharmacol.* 49 (9) (1997) 866–872.
- [13] Y. Tatsumi et al., Therapeutic efficacy of topically applied KP-103 against experimental tinea unguium in guinea pigs in comparison with amorolfine and terbinafine, *Antimicrob. Agents Chemother.* 46 (12) (2002) 3797–3801.
- [14] L.V. Kerai, S. Hilton, S. Murdan, UV-curable gel formulations: potential drug carriers for the topical treatment of nail diseases, *Int. J. Pharm.* 492 (1–2) (2015) 177–190.
- [15] K. Sugiura et al., The low keratin affinity of efinaconazole contributes to its nail penetration and fungicidal activity in topical onychomycosis treatment, *Antimicrob. Agents Chemother.* 58 (7) (2014) 3837–3842.
- [16] J.H. Hildebrand, R.L. Scott, *The Solubility of Nonelectrolytes*, third ed., Reinhold, New York, 1950.
- [17] C.M. Hansen, Solubility parameters – an introduction, in: C.M. Hansen (Ed.), *Hansen Solubility Parameters A User's Handbook*, CRC Press, Boca Raton, US, 2007.
- [18] A.F.M. Barton, *Handbook of Solubility Parameters and Other Cohesive Parameters*, CRC Press, Boca Raton, 1991.
- [19] B.C. Hancock, P. York, R.C. Rowe, The use of solubility parameters in pharmaceutical dosage form design, *Int. J. Pharm.* 148 (1) (1997) 1–21.
- [20] M.A. Mohammad, A. Alhalaweh, S.P. Velaga, Hansen solubility parameter as a tool to predict cocrystal formation, *Int. J. Pharm.* 407 (1–2) (2011) 63–71.
- [21] J. Breitkreutz, Prediction of intestinal drug absorption properties by three-dimensional solubility parameters, *Pharm. Res.* 15 (9) (1998) 1370–1375.
- [22] F.J. Navarro-Lupin, P. Bustamante, B. Escalera, Relationship between swelling of hydroxypropylmethylcellulose and the Hansen and Karger partial solubility parameters, *J. Pharm. Sci.* 94 (7) (2005) 1608–1616.
- [23] H.P. Baden, Physical properties of nail, *J. Invest. Dermatol.* 55 (2) (1970) 115–122.
- [24] H.P. Baden, L.A. Goldsmith, B. Fleming, Comparative study of physicochemical properties of human keratinised tissues, *Biochim. Biophys. Acta* 322 (2) (1973) 269–278.
- [25] O.G. Martinsen, S. Grimnes, S.H. Nilsen, Water sorption and electrical properties of a human nail, *Skin Res. Technol.* 14 (2) (2008) 142–146.
- [26] C. Barba et al., Water sorption of nails treated with wool keratin proteins and peptides, *J. Therm. Anal. Calorim.* 104 (1) (2011) 323–329.
- [27] C.M. Hansen, Hansen solubility parameters, in: *A User's Handbook*, CRC Press, Boca Raton, 2007, p. 519.
- [28] P. Fleckman, Basic science of the nail unit, in: R.K. Scher, C.R. Daniel (Eds.), *Nails: Therapy, Diagnosis, Surgery*, WB Saunders, Philadelphia, 1997, pp. 37–54.
- [29] M. Helmdach et al., Age and sex variation in lipid composition of human fingernail plates, *Skin Pharmacol. Appl. Skin Physiol.* 13 (2) (2000) 111–119.
- [30] S. Murdan, The nail: anatomy, physiology, diseases, and treatment, in: S.N. Murthy, H. Maibach (Eds.), *Topical Nail Products and Ungual Drug Delivery*, CRC Press, Boca Raton, 2012, pp. 1–36.
- [31] M. Gniadecka et al., Structure of water, proteins, and lipids in intact human skin, hair, and nail, *J. Invest. Dermatol.* 110 (4) (1998) 393–398.
- [32] C. Barba et al., Water content of hair and nails, *Thermochim. Acta* 494 (1–2) (2009) 136–140.
- [33] R.H. Khengar et al., Nail swelling as a pre-formulation screen for the selection and optimisation of ungual penetration enhancers, *Pharm. Res.* 24 (2007) 2207–2212.
- [34] M.A. Repka, P.K. Mididoddi, S.P. Stodghill, Influence of human nail etching for the assessment of topical onychomycosis therapies, *Int. J. Pharm.* 282 (1–2) (2004) 95–106.
- [35] M.A. Repka et al., Nail morphology studies as assessments for onychomycosis treatment modalities, *Int. J. Pharm.* 245 (1–2) (2002) 25–36.
- [36] S.R.K. Vaka et al., A platform for predicting and enhancing model drug delivery across the human nail plate, *Drug Dev. Ind. Pharm.* 37 (1) (2011) 72–79.
- [37] W.S. Chiu et al., Molecular diffusion in the human nail measured by stimulated Raman scattering microscopy, *Proc. Natl. Acad. Sci. U.S.A.* 112 (25) (2015) 7725–7730.
- [38] H.B. Gunt, M.A. Miller, G.B. Kasting, Water diffusivity in human nail plate, *J. Pharm. Sci.* 96 (12) (2007) 3352–3362.
- [39] D.K. Stern et al., Water content and other aspects of brittle versus normal fingernails, *J. Am. Acad. Dermatol.* 57 (1) (2007) 31–36.
- [40] M. Egawa, Y. Ozaki, M. Takahashi, In vivo measurement of water content of the fingernail and its seasonal change, *Skin Res. Technol.* 12 (2) (2006) 126–132.
- [41] K.A. Smith, J. Hao, S.K. Li, Effects of organic solvents on the barrier properties of human nail, *J. Pharm. Sci.* 100 (10) (2011) 4244–4257.
- [42] S. Wessel et al., Hydration of human nails investigated by NIR-FT-Raman spectroscopy, *Biochim. Biophys. Acta-Protein Struct. Mol. Enzymol.* 1433 (1–2) (1999) 210–216.
- [43] P.C.M. van de Kerkhof et al., Brittle nail syndrome: a pathogenesis-based approach with a proposed grading system, *J. Am. Acad. Dermatol.* 53 (4) (2005) 644–651.
- [44] H.B. Gunt, G.B. Kasting, Effect of hydration on the permeation of ketoconazole through human nail plate in vitro, *Eur. J. Pharm. Sci.* 32 (4–5) (2007) 254–260.
- [45] S.N. Murthy et al., TransScreen-N (TM): method for rapid screening of transungual drug delivery enhancers, *J. Pharm. Sci.* 98 (11) (2009) 4264–4271.
- [46] P. Chouhan, T.R. Saini, Hydration of nail plate: a novel screening model for transungual drug permeation enhancers, *Int. J. Pharm.* 436 (1–2) (2012) 179–182.
- [47] C.M. Hansen, T.S. Poulsen, Hansen solubility parameters – biological materials, in: C.M. Hansen (Ed.), *Hansen Solubility Parameters A User's Handbook*, CRC Press, Boca Raton, US, 2007, pp. 269–292.
- [48] H.P. Baden, J. Kubilus, Fibrous proteins of bovine hoof, *J. Invest. Dermatol.* 81 (3) (1983) 220–224.
- [49] K.A. Walters, G.L. Flynn, J.R. Marvel, Physicochemical characterization of the human nail – permeation pattern for water and the homologous alcohols and differences with respect to the stratum-corneum, *J. Pharm. Pharmacol.* 35 (1) (1983) 28–33.
- [50] D. Cooper, T.T. Sun, Monoclonal-antibody analysis of bovine epithelial keratins – specific pairs as defined by coexpression, *J. Biol. Chem.* 261 (10) (1986) 4646–4654.
- [51] S. Murdan et al., In vivo measurement of the surface energy of human fingernail plates, *Int. J. Cosmet. Sci.* 34 (3) (2012) 257–262.
- [52] S. Nicoli et al., Comparison of physico-chemical properties of human nail and bovine hoof in skin and formulation 3rd symposium & skin forum, in: 10th Annual Meeting, Versailles, France, 2009.
- [53] P. Fleckman, Structure and function of the nail unit, in: R. Scher, C.R. Daniel III (Eds.), *Nails. Diagnosis Therapy Surgery*, Elsevier Saunders, 2005, pp. 13–25.
- [54] J.B. Hamilton, H. Terada, G.E. Mestler, Studies of growth throughout the lifespan in Japanese: Growth and size of nails and their relationship to age, sex, heredity, and other factors, *J. Gerontol.* 10 (4) (1955) 401–415.
- [55] S. Ohgitani et al., Nail calcium and magnesium content in relation to age and bone mineral density, *J. Bone Mineral Metab.* 23 (4) (2005) 318–322.
- [56] S. Murdan, G. Milcovich, G.S. Goriparthi, An assessment of the human nail plate pH, *Skin Pharmacol. Physiol.* 24 (4) (2011) 175–181.
- [57] S. Murdan, D. Hinsu, M. Guimier, A few aspects of transonychia nail loss (TOWL): inter-individual, and intra-individual inter-finger, inter-hand and inter-day variabilities, and the influence of nail plate hydration, filing and varnish, *Eur. J. Pharm. Biopharm.* 70 (2) (2008) 684–689.
- [58] E. Widjaja, R.K.H. Seah, Use of Raman spectroscopy and multivariate classification techniques for the differentiation of fingernails and toenails, *Appl. Spectrosc.* 60 (3) (2006) 343–345.
- [59] G. Midgley et al., Mycology of nail disorders, *J. Am. Acad. Dermatol.* 31 (3) (1994) S68–S74.
- [60] E. Reiss, H.J. Shadomy, G.M. Lyon III, *Fundamental Medical Mycology*, Wiley-Blackwell, New Jersey, 2012.
- [61] B. Hossin, The rational design of an antifungal nail lacquer using the Hansen solubility parameter concept, in: UCL School of Pharmacy, University College London, London, 2015.